REMARKS

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and the following remarks.

The Declarant's signature for the 37 C.F.R. § 1.132 declaration was not able to be obtained before the due date. The fully executed declaration will be provided shortly.

I. Status of the Claims

Claims 1-23, 25 and 29 were pending in the application, with claims 1 and 23 being the independent claims. Claim 1 is currently amended and claim 30 is newly added. Thus, upon entry of this paper, claims 1-23, 25 and 29-30 are currently pending and under active consideration.

Support for the term "wherein the proportion of the label-isotope of at least 50 metabolites of the biological sample is increased to at least 80% of the total of all isotopes of the element" can be found throughout the specification. Specifically, support can be found on page 9, lines 21 to 26 and page 10 lines 25 to 29 of the specification.

II. Claim Rejection Under 35 U.S.C. § 103(a)- Lee

The Office Action, at pages 4-6, rejects claims 1, 5-8, 10-13 and 20-22 under 35 U.S.C. § 103(a) as allegedly being obvious over US Patent Application Publication No. 2003/0180710 A1 to Lee *et al.* ("Lee"). Applicants respectfully traverse this ground of rejection.

A. Current Obviousness Standard

The Supreme Court recently reaffirmed the Graham factors for determining obviousness in *KSR Int'l Co. v. Teleflex Inc.* (550 U.S. 398 (2007)). The Graham factors, as outlined by the Supreme Court in *Graham et al. v. John Deere Co. of Kansas City et al.*, 383 U.S. 1 (1966), are: 1) determining the scope and contents of the prior art; 2) ascertaining the differences between the claimed invention and the prior art; 3) resolving the level of ordinary skill in the pertinent art; and 4) evaluating evidence of secondary consideration. The Supreme Court recognized that a showing of "teaching, suggestion, or motivation" to

combine the prior art to meet the claimed subject matter could provide a helpful insight in determining whether the claimed subject matter is obvious under 35 U.S.C. § 103(a), and held that the proper inquiry for determining obviousness is whether the improvement is more than the predictable use of prior art elements according to their established functions. The Court noted that it is "important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the [prior art] elements" in the manner claimed, and specifically stated:

> Often, it will be necessary . . . to look to interrelated teachings of multiple patents; the effects of demands known to the design community or present in the marketplace; and the background knowledge possessed by a person having ordinary skill in the art, all in order to determine whether there was ***an apparent reason to combine the known elements in the fashion claimed*** by the patent at issue. To facilitate review, this analysis should be made explicit.

KSR Int'l Co. v. Teleflex Inc., 550 U.S. 398, 418 (2007) (emphasis added). As discussed below, the cited art cannot render the claimed invention obvious.

B. Isotope of Carbon

As an initial matter, the Office states that claim 1 requires that cells take up a labeled compound such that its metabolites are saturated with the isotope of the compound. Additionally, the Office states "Since ^{12}C is an isotope of carbon, the claim as written may require revision to clarify applicant's intent." (Office Action, page 2)

Applicants define the term "isotopic labeling" on page 9 line 21 to page 10 line 6. Specifically, applicants define "isotopic labeling" to "be understood to refer to compounds that are labeled with an isotope that is not the main isotope of the element of said isotope." (See specification page 9 lines 21-22). Thus, the "isotopically labeled metabolizable compound" would not include ^{12}C, because ^{12}C is the main isotope of the element.

C. The Proposed Amendments Traverse the Rejection

The Office Action, at page 3, contends "Lee teaches methods of studying metabolism and that precursors may be partially or uniformly labeled. Precursors are metabolized, whether they are partially or uniformly labeled, and metabolites can be detected and

quantified in an analysis of isotopomers, which is also taught by Lee." (Office Action, page 3).

In an effort to advance prosecution of this application and without acquiescing to the propriety of this rejection, Applicants have amended the claims to bring out the distinction between conventional flux techniques and the claimed invention. Specifically, it would not be possible to detect influences of drugs or substances on the metabolism as intended by the method of Lee if Lee employed the strategy of the amended claimed invention. (See also Section III(D) below)

D. Combination of the Claimed Elements Would Render Lee Inoperable

The Examiner is reminded that "[i]f proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. *In re Gordon*, 733 F.2d 900, 211 USPQ 1125 (Fed. Cir. 1984)." MPEP 2143.01 Section V.

Lee teaches the determination of the dynamic distribution of the label isotope in specific compounds for a mechanistic purpose. (*See Declaration to be provided shortly*) Specifically, Lee measures the 13-C/12-C ratio of certain metabolites at certain positions within these individual metabolites. Lee uses "smartly labelled" compounds, in contrast to Applicants' uniformly labelled compounds. Lee uses only one sample (no combination of labelled and unlabelled samples) and measures those samples at specific time points to determine the dynamics of these metabolites. In determining the 13-C/12-C ratio of certain metabolites Lee does not claim to perform absolute quantification, rather Lee shows how to quantify the relative changes of the labelling ratio. (*See Declaration to be provided shortly.*)

Lee discloses "smartly labelled" precursors at known positions. In contrast, the claimed invention uses saturated uniformly labelled compounds. As is known to one of skill in the art, it is not possible to obtain a saturated uniformly labelled sample, independently of the duration of labelling. (*See Declaration to be provided shortly.*) Thus, the purpose of Lee's method is to use "smartly labelled" precursors to explore **individual** metabolic

pathways. (*See Declaration to be provided shortly.*) This is clearly in contrast to the claimed invention.

For determining the dynamics of distribution of the labelled atoms of the precursor, also known as flux measurements, it is necessary to perform **time dependent** measurements. A uniformly and saturated labelled sample without any further change would not provide more information than a completely natural and unlabeled sample. As such, the claimed invention would not provide any dynamic or flow information.

Such a uniformly and saturated labelled sample without any further change after being produced is only useful if combined with an unlabeled sample (of natural 13C content) for use as an internal standard for precise, absolute quantification, as described in the claimed invention. (*See Declaration to be provided shortly.*) **Lee does not combine samples and does not perform any absolute quantification**.

In contrast to the claimed invention, **Lee would not be operable if he used uniformly and saturated labelled samples**. Lee only discloses a method of determining dynamics (time dependant distribution) and pathways (specifically labelled compounds and position of labelling within a compound). If Lee were to use uniformly and saturated labelled samples, Lee could not determine the dynamics or pathways involved (*See Declaration to be provided shortly.*), thereby frustrating the very purpose of Lee's invention.

As such, Lee's method is completely different from the claimed invention. Applicants surprisingly found that a uniformly and saturated labelled sample, when combined with an unlabeled sample leads to an unexpectedly good system for the absolute and comprehensive quantification of a metabolome, and even of compounds without available pure isotopically labelled standards. Both the labelled and unlabeled sample described by applicants, do not rely on any time dependent changes. In contrast, Lee's method requires the measurement of time dependent effects to determine the dynamics of the labelled compound. (*See Declaration to be provided shortly.*)

Therefore, Lee is completely distinct from the claimed invention. Additionally, if one of skill in the art tried to apply the teachings disclosed by applicant to Lee's method, Lee's

method would be rendered inoperable. As such, Applicants respectfully request reconsideration and withdrawal of the rejection.

III. Claim Rejection Under 35 U.S.C. § 103-Lee in view of Abramson

The Office Action, at pages 7-8, rejects claims 2-4 under 35 U.S.C. § 103(a) as allegedly being obvious over US Patent Application Publication No. 2003/0180710 A1 to Lee *et al.* ("Lee") in view of US Patent Application Publication No. 2003/0077572 A1 to Abramson *et al.* ("Abramson"). Applicants respectfully traverse this ground of rejection.

Abramson teaches the saturated, uniform labelling of a sample and the combination with an unlabelled, but treated, sample for quantification purpose. (*See Declaration to be provided shortly.*) Therefore, Abramson does not teach a method for analysing the metabolites of a biological sample which comprises quantitatively determining one or more metabolites in said sample in a way that said quantitative analysis resolves isotopic mass differences of the metabolites themselves or of at least one specific mass spectral fragment per metabolite originating from and being representative for the particular metabolite. (*See Declaration to be provided shortly.*) Specifically, Abramson only measures the isotopic mass difference of the combustion products but not the metabolites. (*See Declaration to be provided shortly.*)

For the quantification of a complex sample, Abramson combines an established method, isotope ratio mass spectrometry (IRMS), with chromatography isotope dilution analysis. (*See Declaration to be provided shortly.*) In IRMS, the sample is usually decomposed to unspecific small compounds, such as CO_2 for all carbon atoms of the sample. (*See Declaration to be provided shortly.*) Specifically, Abramson uses CRIMS, a special interface for combustion, to decompose the sample. (*See Declaration to be provided shortly.*) This type of analysis is a convenient, simple and easy way to apply isotope dilution analysis, however, the output contains only **two-dimensional data.** (*See Declaration to be provided shortly.*) Specifically, the obtained isotope ratio compared against the chromatographic analysis results in the direct quantitative measure for compositional changes of the sample after treatment. (*See Declaration to be provided shortly.*)

One key structural limitation of Abramson is based on the combustion process necessary to decompose the sample. (*See Declaration to be provided shortly.*) Specifically, all structural information of the metabolites is lost because the sample is decomposed via combustion. (*See Declaration to be provided shortly.*) As such, Abramson characterizes metabolites using only chromatographic retention time. (*See Declaration to be provided shortly.*) Thus, **two dimensional** chromatography, as described in Abramson, is **insufficient for calculating individual quantitative results on thousands of compounds**. (*See Declaration to be provided shortly.*) Therefore, Abramson is not suited for complex metabolomic analysis. (*See Declaration to be provided shortly.*)

Additionally Abramson combines an ionising mass spectrometer in parallel to CRIMS, but only for analyte identification purposes. Furthermore, Abramson requires a fraction collection step before the analyte can be identified. However, these steps do not improve the poor selectivity and resolution of metabolites for the quantification. (*See Declaration to be provided shortly.*)

In contrast, Applicants found **that if the mass spectrometer is coupled directly to the chromatography**, instead of the CRIMS (Abramson), unexpectedly comprehensive, precise and selective quantitation results can be obtained. Additionally, Applicants have combined this technique with comprehensive isotope dilution (combination of a uniformly and saturatedly labelled sample with an unlabeled one) for the analysis of complex mixtures. Thus, for metabolomics, the output generated by the claimed invention contains unexpectedly comprehensive, precise and selective quantitation results.

Applicants have added a **third dimension** of analysis not present in Abramson. Specifically, this third dimension consists of analysis of the molecular and fragment ions masses. The third dimension increases the selectivity in a manner so that completely coeluting compounds with known and different mass spectra can easily be resolved. Surprisingly, even partly coeluting metabolites with unknown mass spectra can be resolved by deconvolution. (*See Declaration to be provided shortly.*) This is a crucial improvement for metabolomics because never before could one of skill in the art analyze a high number of compounds. Specifically, the quantification quality of previous methods are limited because

the high number of compounds were poorly resolved when using only chromatography (Abramson).

Applicants use the mass spectral monitoring of the highly selective molecular and fragment ion masses in parallel. This strongly improves metabolite resolution and therewith the precision of the assignment of the isotope ratio of particular metabolites. Said isotope ratio is the measure for quantitation of the particular metabolites in the claimed method. Additionally, this works for the combination of an uniformly and saturatedly labelled complex sample with an identically complex unlabelled sample. Finally, unlike previous methods, Applicants' method allows for the comprehensive, precise and quantitative measurements necessary for metabolomics. (*See Declaration to be provided shortly.*)

In sum, Abramson does not teach a method for analysing the metabolites of a biological sample which comprises quantitatively determining one or more metabolites in said sample in a way that said quantitative analysis resolves isotopic mass differences of the metabolites themselves or of at least one specific mass spectral fragment per metabolite originating from and being representative for the particular metabolite. Specifically, Abramson only measures the isotopic mass difference of the combustion products but not the metabolites.

Lee does not cure the deficiencies of Abramson. Specifically, the combination of Lee's method with Abramson's disclosure would not make the finding obvious to a person skilled in the art. This is because Lee's use of mass spectrometry for isotope dilution analysis **does not have the intention or function of absolute quantification** and **does not use the combination of samples** for this purpose. As discussed in Section III(D) above, Lee's method and objective is clearly **contrary** to the challenge of the claimed invention that it could not and would not be taken into account for solving the problem in combination with the disclosure of Abramson.

As such, Lee does not remedy the deficiencies of Abramson described above. In fact, Abramson discloses differential labeling of two cell populations, each labeled to a different extent, but fails in precisely quantifying the plurality of the metabolites of a metabolome

selectively and separately. Thus, Abramson fails to teach or suggest the claimed invention. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

E. The Rejection Over Lee in view of Kasper

The Office Action, at pages 6-7, rejects claim 14 under 35 U.S.C. § 103(a) as allegedly being obvious over US Patent Application Publication No. 2003/0180710 A1 to Lee *et al.* ("Lee") in view of US Patent Application Publication No. 2005/0112706 A1 to Kasper. Applicants respectfully traverse this ground of rejection.

Kasper discloses methods for determining androgen responsiveness in a sample using bioassays. Kasper fails to teach or suggest cell labeling, let alone saturated labeling. Thus, Kasper fails to remedy the deficiencies of Lee.

Accordingly, the rejection is improper and should be withdrawn.

F. The Rejection Over Lee in view of Birkemeyer

The Office Action, at pages 7-8, rejects claims 16-17 under 35 U.S.C. § 103(a) as allegedly being obvious over US Patent Application Publication No. 2003/0180710 A1 to Lee *et al.* ("Lee") in view of Birkemeyer *et al.* 2003 J. Chromatography A 993: 89 ("Birkemeyer"). Applicants respectfully traverse this ground of rejection.

Birkemeyer discloses gas chromatography analysis of phytohormones. The reference fails to teach or suggest isotope labeling, let alone saturated labeling, and thus fails to remedy the deficiencies of Lee described above.

Thus, the rejection is improper. Reconsideration and withdrawal of this ground of rejection are therefore respectfully requested.

G. The Rejection Over Lee in view of Hellerstein-APEM

The Office Action, at pages 8-9, rejects claims 18-19 under 35 U.S.C. § 103(a) as allegedly being obvious over US Patent Application Publication No. 2003/0180710 A1 to Lee *et al.* ("Lee") in view of MK Hellerstein and RA Neese 1999 *American J. Physiol. Endocr. Metab.* 276: 1146- 1170 ("Hellerstein-APEM"). Applicants respectfully traverse this ground of rejection.

Hellerstein-APEM fails to remedy the deficiencies of Lee described above, as the reference provides a review of mass isotopomer distribution and no saturated labeling is taught or suggested by Hellerstein-APEM. Accordingly, the rejection is improper and should be withdrawn.

H. The Rejection Over Lee in view of Hellerstein

The Office Action, at page 9, rejects claim 25 under 35 U.S.C. § 103(a) as allegedly being obvious over US Patent Application Publication No. 2003/0180710 A1 to Lee *et al.* ("Lee") in view of US Patent Application Publication No. 2004/00811994 A1 to Hellerstein ("Hellerstein"). Applicants respectfully traverse this ground of rejection.

Hellerstein discloses biochemical methods for assessing metabolic fitness with time dependent isotopic label distribution (flux) measurements of specific metabolite classes (RNA, DNA, proteins and phospholipids), similar to Lee, using low labeling grades and performing no absolute quantification. The reference fails to teach or suggest cell labeling, and thus fails to remedy the deficiencies of Lee described above. Accordingly, the rejection is improper and should be withdrawn.

I. The Rejection Over Lee in view of Evans

The Office Action, at pages 9-10, rejects claim 23 under 35 U.S.C. § 103(a) as allegedly being obvious over US Patent Application Publication No. 2003/0180710 A1 to Lee *et al.* ("Lee") in view of US Patent No. 5,532,206 to Evans *et al.* ("Evans"). Applicants respectfully traverse this ground of rejection.

Evans does not remedy the deficiencies of Lee described above as the patent discloses application of C-16,17- dihydro gibberellin to plants, however no isotope labeling.

Accordingly, the rejection is improper and should be withdrawn.

J. The Rejection Over Lee in view of Evans and Further in view of Hellerstein

The Office Action, at page 10, rejects claim 29 under 35 U.S.C. § 103(a) as allegedly being obvious over US Patent Application Publication No. 2003/0180710 A1 to Lee *et al.* ("Lee") in view of US Patent No. 5,532,206 to Evans *et al.* ("Evans") and further in view of US Patent Application Publication No. 2004/00811994 A1 to Hellerstein ("Hellerstein"). Applicants respectfully traverse this ground of rejection.

As discussed above, none of the cited references teaches or suggest the claimed invention. Thus, the rejection is improper. Reconsideration and withdrawal of this ground of rejection are therefore respectfully requested.

CONCLUSION

All of the stated grounds of objection and rejection have been properly traversed or rendered moot. Thus, the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing or a credit card payment form being unsigned, providing incorrect information resulting in a rejected credit card transaction, or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicants hereby petition for such extension under 37 C.F.R. § 1.136 and authorize payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

By _____

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